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EZ Extraction Solution

Instruction Manual

(Version 1, August 2005)

Cat. No.: GN-EZO-001 50ml

Cat. No.: GN-EZO-002 200ml

Store at 2 ~ 8 ℃

EZ EXTRACTION Solution (*EZ*ol)

User's Manual

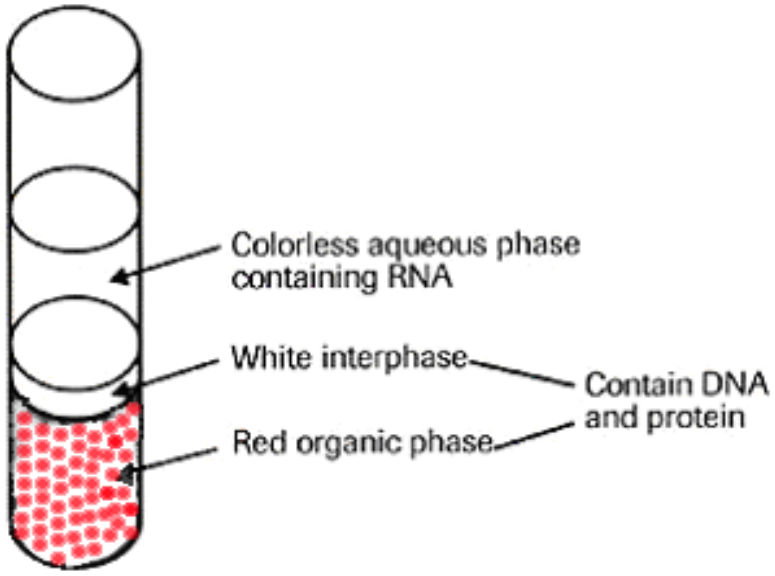
Reagent required, but not supplied:

1. Chloroform
2. Isopropyl alcohol
3. 75% Ethanol (in DEPC-treated water)
4. RNase-free water or 0.5% SDS solution. To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethypyrocarbonate (DEPC) to 0.1%(v/v). Let stand overnight and autoclave. SDS solution must be prepared using DEPC-treated, autoclaved water.

Precautions for Preventing RNase Contamination:

- RNase can be introduced accidentally into RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to be inhibited, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.
- Always wear disposable gloves. Skin often contains bacteria that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
- Use sterile, disposable plasticware and automatic pipettors reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettors) can be rich sources of RNase.
- In the presence of ***EZ***ol , RNA is protected from RNase contamination. Downstream sample handing requires that nondisposable glassware or plasticware is RNase-free. Glassware can be baked at 150℃ for 4 hours, and plasticware can be soaked for 10 minutes in 0.5M NaOH, rinsed thoroughly with water, and autoclaved.

INSTRUCTION FOR RNA ISOLATION:



Prepare cells or tissues

↓

Add ***EZ***ol

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Separate phase (Add chloroform)

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Transfer the upper aqueous phase to a new tube

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Precipitate RNA

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Wash and solubilize RNA pellet

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Store at -70℃

CAUTION: When working with ***EZ***ol, wear gloves and eye protection (shield, safety goggles). Avoid contacting with skin or clothing. Handle in a chemical fume hood. Avoid breathing vapor.

Note: Unless otherwise stated, the procedure is carried out at 15~30℃, and ***EZ***ol is stored at 2~8℃.

1. HOMOGENIZATION

a. Tissues-- Homogenize tissue samples in 1 ml of ***EZ***ol per 50-100 mg of tissue using a glass-Teflon or power homogenizer (Polytron, or Tekmar's TISSUMIZER or equivalent). The sample volume should not exceed 10% of the volume of ***EZ***ol used for homogenization.

b. Cells Grown in Monolayer-- Lyse cells directly in culture dish by adding 1 ml ***EZ***ol to a 3.5 cm diameter dish, and pass the cell lysate through a pipette several times. The amount of ***EZ***ol added is based on the area of the culture dish (1 ml per $10cm^2$) and not on the number of cells present. An insufficient amount of ***EZ***ol may result in contamination of the isolated RNA with DNA.

c. Cells Grown in Suspension-- Pellet cells by centrifugation. Lyse cells in ***EZ***ol by repetitive pipetting. Use 1 ml of ***EZ***ol per 5~10 X 10^6 of animal, plant or yeast cells, or per 1x 10^7 bacteria cells. Washing cells before addition of ***EZ***ol should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. OPTIONAL

An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fatty tissue, and tuberous parts of plants, 12,000 rpm for 10 minutes at 2~8℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA pellet, while the supernatant contains RNA. In samples from fatty tissues, an excess of fat collects as a upper layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

3. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15~30℃ to allow the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of ***Ez***ol. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15~30℃ for 3~5 minutes. Centrifuge the samples at not more than 12,000 rpm for 15 minutes at 2~8℃. Following centrifugation, the mixture separates into a lower, phenol-chloroform phase, a cloudy white interphase, and a colorless upper aqueous phase. RNA remains exclusively in the upper aqueous phase. The volume of the aqueous phase is about 60% of the volume of ***EZ***ol used for homogenization.

4. RNA PRECIPITATION

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of ***EZ***ol used for the initial homogenization. Incubate samples for 10 minutes at 15~30℃ and then centrifuge at 12,000 rmp for 10 minutes at 2~10℃ to form a gel-like pellet at the bottom of the tube.

5. RNA WASH

Remove the supernatant. To wash the RNA pellet, add at least 1 ml of 75% ethanol per 1 ml of ***EZ***ol used for the initial homogenization. Mix the sample by vortexing and centrifuge at not more than 7,500 rpm for 5 minutes at 2-8℃.

6. REDISSOLVING THE RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/A280 ratio<1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution through a pipette tip several times, and incubate at 55-60℃for 10 minutes (do not use SDS solution when RNA will be used in subsequent enzymatic reactions.) RNA can also be dissolved in 100% formamide (deionized) and stored at -70℃.

TROUBLESHOOTING GUIDE

1. Expected yield of RNA per mg of tissues or 1x 10^6 cultured cells

Liver and spleen	6-10μg	Kidney	3-4μg
Skeletal muscles and brain	1-1.5μg	Placenta	1-4μg
Epithelial cells	8-5μg	Fibroblasts	5-7μg

2. Low yield

- Incomplete homogenization or lysis of samples.
- Final RNA pellet incompletely redissolved.

3. **A260/A280 ratio <1.65**
   - RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase absorbance at 280nm. (See Wilfinger, W. et al, Biotechniques 22:474-481. and Fox, D.K (1998) Focus 20:2 p.37).
   - Insufficient amount of ***EZ***ol was used in sample homogenization.
   - Following homogenization, samples did not be placed at room temperature for 5 minutes.
   - The aqueous phase was contaminated with the phenol phase.
   - Incomplete dissolution of the final RNA pellet.

4. **RNA degradation**
   - Tissues did not be immediately processed or frozen after removal from animals.
   - Samples used for isolation, or the isolated RNA preparations were stored at -5~ -20℃, instead of -60~ -70℃.
   - Cultured Cells were dispersed by trypsin digestion.
   - Aqueous solutions or tubes were not RNase-free.
   - Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

5. **RNA contamination**
   - Samples homogenized in too less volume of ***EZ***ol.
   - Samples used for the isolation contained organic solvents (e.g, ethanol, DMSO), strong buffer, or alkaline solution.
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6. **Proteoglycan and polysaccharide contamination**
   - The following modification of the RNA precipitation removes these contamination compounds from the isolated RNA. Add to the aqueous phase 0.25 ml of isopropyl alcohol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of ***EZ***ol used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in solution. A combination of modified precipitation step with an additional centrifugation of the initial homogenate is required to isolate pure RNA from plant materials containing a very high level of polysaccharides.

Storage condition: 2~8 ℃

EZol has demonstrated stability of 12 months when store at RT. However, we recommend to store at 2-8℃ for optimal performance.

Ordering information

- Cat. No. EZO-001 50ml
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